WEST Search History

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DATE: Thursday, August 09, 2007

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=OR	
☐	L36	L35 same promoter	5
☐	L35	L34 same (bacillus or subtilis)	52
☐	L34	ribosomal near rna near gene	1095
☐	L33	ribosmal near rna near gene	0
☐	L32	rna near gene near set	8
☐	L31	rrrno	15
☐	L30	l29 and ogasawara$	5
☐	L29	L27 near25 (bacill$ or subtilis!)	117
☐	L28	L27 same (bacill$ or subtilis!)	830
☐	L27	(rrno or rrn or ribosome or ribosomal or rrna) near3 (p1 or p2 or operon or promoter)	9917
☐	L26	L4 same (foreign or heterologous or heter-ologous or recombinant$ or plasmid or vector or cassette or (delivery near2 system))	30
☐	L25	l4	93
☐	L24	L20 and (clostrid$ or tetan$)	62
☐	L23	L20 and (tetan$)	36
☐	L22	L20 and (clostrid$ or tetan$)	62
☐	L21	L20 and (clostrid$ or fragment or tetan$)	269
☐	L20	l14 and $toxin.clm. not l16 not l17	401
☐	L19	l15 and $toxin not l16 not l17	0
☐	L18	l15 and $toxin.clm. not l16 not l17	0
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☐	L16	L14 same tetan$.clm.	22
☐	L15	L14 and tetan$.clm.	48
☐	L14	($spor$ or sporu$ or $spore).clm.	147648
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☐	L11	($spor$ or sporu$ or $spore).ti.	4280
		L9 same (foreign or heterologous or heter-ologous or recombinant$ or	

☐	L10	plasmid or vector or cassette or (delivery near2 system)).clm.	53
☐	L9	tetanus!.clm. same $toxin.clm.	306
☐	L8	l2 and $toxin.clm.	64
☐	L7	l2 and (tetanus or fragment-c or c-fragment or Hc or bindingdomain or cellbinding).clm.	0
☐	L6	l2 and (tetanus or fragment-c or c-fragment or heavy or Hc or bindingdomain or cellbinding).clm.	13
☐	L5	L4 and (foreign or heterologous or heter-ologous or recombinant$ or plasmid or vector or cassette or (delivery near2 system))	89
☐	L4	(rrno or rrn) near3 (p1 or p2 or operon)	93
☐	L3	L2 and ($spor$ or sporu$ or $spore).clm.	38
☐	L2	host.clm. same bacill$.clm.	759
☐	L1	tetanus! near10 (spore$ or $spore)	48

END OF SEARCH HISTORY

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Thursday, August 09, 2007

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☐	L40	L37 and (okamoto$ or vold$)	0
☐	L39	L37 and subtilis	7
☐	L38	L37 and 168	4
☐	L37	goldman.in. and spore	20
☐	L36	L35 same promoter	5
☐	L35	L34 same (bacillus or subtilis)	52
☐	L34	ribosomal near rna near gene	1095
☐	L33	ribosmal near rna near gene	0
☐	L32	rna near gene near set	8
☐	L31	rrrno	15
☐	L30	l29 and ogasawara$	5
☐	L29	L27 near25 (bacill$ or subtilis!)	117
☐	L28	L27 same (bacill$ or subtilis!)	830
☐	L27	(rrno or rrn or ribosome or ribosomal or rrna) near3 (p1 or p2 or operon or promoter)	9917
☐	L26	L4 same (foreign or heterologous or heter-ologous or recombinant$ or plasmid or vector or cassette or (delivery near2 system))	30
☐	L25	l4	93
☐	L24	L20 and (clostrid$ or tetan$)	62
☐	L23	L20 and (tetan$)	36
☐	L22	L20 and (clostrid$ or tetan$)	62
☐	L21	L20 and (clostrid$ or fragment or tetan$)	269
☐	L20	l14 and $toxin.clm. not l16 not l17	401
☐	L19	l15 and $toxin not l16 not l17	0
☐	L18	l15 and $toxin.clm. not l16 not l17	0
☐	L17	l15 not l16	26
☐	L16	L14 same tetan$.clm.	22
☐	L15	L14 and tetan$.clm.	48

☐	L14	($spor$ or sporu$ or $spore).clm.	147648
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☐	L12	L11 and tetan$	19
☐	L11	($spor$ or sporu$ or $spore).ti.	4280
☐	L10	L9 same (foreign or heterologous or heter-ologous or recombinant$ or plasmid or vector or cassette or (delivery near2 system)).clm.	53
☐	L9	tetanus!.clm. same $toxin.clm.	306
☐	L8	l2 and $toxin.clm.	64
☐	L7	l2 and (tetanus or fragment-c or c-fragment or Hc or bindingdomain or cellbinding).clm.	0
☐	L6	l2 and (tetanus or fragment-c or c-fragment or heavy or Hc or bindingdomain or cellbinding).clm.	13
☐	L5	L4 and (foreign or heterologous or heter-ologous or recombinant$ or plasmid or vector or cassette or (delivery near2 system))	89
☐	L4	(rrno or rrn) near3 (p1 or p2 or operon)	93
☐	L3	L2 and ($spor$ or sporu$ or $spore).clm.	38
☐	L2	host.clm. same bacill$.clm.	759
☐	L1	tetanus! near10 (spore$ or $spore)	48

END OF SEARCH HISTORY

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LOCUS       NC_000964                144 bp    DNA     linear   BCT 04-JUN-2004
DEFINITION  Bacillus subtilis, complete genome.
ACCESSION   NC_000964 REGION: 14690..14833
VERSION     NC_000964.1  GI:16077068
PROJECT     GenomeProject:76
KEYWORDS    .
SOURCE      Bacillus subtilis subsp. subtilis str. 168
  ORGANISM  Bacillus subtilis subsp. subtilis str. 168
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 144)
  AUTHORS   Kunst,F., Ogasawara,N., Moszer,I., Albertini,A.M., Alloni,G.,
            Azevedo,V., Bertero,M.G., Bessieres,P., Bolotin,A., Borchert,S.,
            Borriss,R., Boursier,L., Brans,A., Braun,M., Brignell,S.C.,
            Bron,S., Brouillet,S., Bruschi,C.V., Caldwell,B., Capuano,V.,
            Carter,N.M., Choi,S.K., Codani,J.J., Connerton,I.F., Danchin,A. et
            al.
  TITLE     The complete genome sequence of the gram-positive bacterium
            Bacillus subtilis
  JOURNAL   Nature 390 (6657), 249-256 (1997)
   PUBMED   9384377
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence was derived from AL009126.
            [WARNING] On Jul 29, 2004 this sequence was replaced by
            gi:50812173.
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//
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INTERNATIONAL SEARCH REPORT

Interna Application No
PCT/ GB 03/00966

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROBINSON K ET AL: "ORAL VACCINATION OF MICE AGAINST TETANUS WITH RECOMBINANT LACTOCOCCUS LACTIS" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 15, no. 7, 15 July 1997 (1997-07-15), pages 653-657, XP001093957 ISSN: 1087-0156 the whole document	11
X	MINTON NIGEL P ET AL: "Chemotherapeutic tumour targeting using clostridial spores." FEMS MICROBIOLOGY REVIEWS, vol. 17, no. 3, 1995, pages 357-364, XP002242524 ISSN: 0168-6445 the whole document	21,22

INTERNATIONAL SEARCH REPORT

Interna Application No
PCT/ 03/00966

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N3/00 C12N15/00 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02 00232 A (MAXYGEN INC ;GOLDMAN STANLEY (US); WHALEN ROBERT G (US); LATHROP S) 3 January 2002 (2002-01-03)	1-8, 10-12, 17,18, 23-31
Y	in particular, examples and claims. the whole document	11
X	WO 01 94599 A (KATAGIHALLIMATH NAINESH ;MUKHERJEE KAKOLI (IN); SMITTSKYDDSINSTITU) 13 December 2001 (2001-12-13)	1-13, 15-20, 23-31
Y	the whole document	11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2003	10/06/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rojo Romeo, E

Form PCT/ISA/210 (second sheet) (July 1992)

Minicell$

2003021599
2003021940 8
2003720 14
2003019908 8
7183105

b35
1988
Wisdom, Russell Lee

Operons
Bacillus

DOCUMENT-IDENTIFIER: US 5571694 A
TITLE: Expression of tetanus toxin fragment C in yeast

CLAIMS:

1. An expression vector which incorporates DNA encoding tetanus toxin fragment C having the amino acid sequence shown in SEQ ID NO:2 and having an increased (G+C)-content relative to the wild-type DNA sequence shown in SEQ ID NO:1 in each of the following regions:

(i) from nucleotide 510 to nucleotide 710,

(ii) from nucleotide 650 to nucleotide 850,

(iii) from nucleotide 800 to nucleotide 1100,

(vi) from nucleotide 900 to nucleotide 1200 and,

(v) from nucleotide 1100 to nucleotide 1356,

the numbers corresponding to those set forth in the sequence of SEQ ID NO:1 and SEQ ID NO:3, so as to allow the production of complete mRNA transcripts in yeast, which vector thereby expresses said fragment C in yeast, wherein said DNA has the sequence shown in SEQ ID NO:3 in each of said regions (i)-(v).

2. A yeast organism transformed with an expression vector which incorporates DNA encoding tetanus toxin fragment C having the amino acid sequence shown in SEQ ID NO:2 and having an increased (G+C)-content relative to the wild-type DNA sequence shown in SEQ ID NO:1 in each of the following regions:

(i) from nucleotide 510 to nucleotide 710,

(ii from nucleotide 650 to nucleotide 850,

(iii from nucleotide 800 to nucleotide 1100,

(vi from nucleotide 900 to nucleotide 1200 and,

(v) from nucleotide 1100 to nucleotide 1356,

the numbers corresponding to those set forth in the sequence of SEQ ID NO:1 and SEQ ID NO:3, so as to allow the production of complete mRNA transcripts in yeast, which vector thereby expresses said fragment C in yeast, wherein said DNA has the sequence shown in SEQ ID NO:3 in each of said regions (i)-(v).

3. A process for the preparation of fragment C of tetanus toxin, which comprises the culturing of a yeast organism transformed with an expression vector which incorporates DNA encoding tetanus toxin fragment C having the amino acid sequence shown in SEQ ID NO:2 and having an increased (G+C)-content relative to the wild-type DNA sequence shown in SEQ ID NO:1 in each of the following regions:

(i) from nucleotide 510 to nucleotide 710,

(ii) from nucleotide 650 to nucleotide 850,

(iii) from nucleotide 800 to nucleotide 1100,

(vi) from nucleotide 900 to nucleotide 1200 and,

(v) from nucleotide 1100 to nucleotide 1356,

the numbers corresponding to those set forth in the sequence of SEQ ID NO:1 and SEQ ID NO:3, so as to allow the production of complete mRNA transcripts in yeast, which vector thereby expresses said fragment C in yeast, wherein said DNA has the sequence shown in SEQ ID NO:3 in each of said regions (i)-(v).

US 20030165538A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0165538 A1

Goldman et al. (43) Pub. Date: Sep. 4, 2003

(54) METHODS AND COMPOSITIONS FOR DEVELOPING SPORE DISPLAY SYSTEMS FOR MEDICINAL AND INDUSTRIAL APPLICATIONS

(75) Inventors: **Stanley Goldman**, Walnut Creek, CA (US); **Stephanie J. Lathrop**, Sunnyvale, CA (US); **Pascal F. Longchamp**, East Palo Alto, CA (US); **Robert G. Whalen**, Foster City, CA (US)

Correspondence Address:
MAXYGEN, INC.
INTELLECTUAL PROPERTY DEPARTMENT
515 GALVESTON DRIVE
RED WOOD CITY, CA 94063 (US)

(73) Assignee: **Maxygen Incorporated**

(21) Appl. No.: **09/892,208**

(22) Filed: **Jun. 26, 2001**

Related U.S. Application Data

(60) Provisional application No. 60/214,161, filed on Jun. 26, 2000.

Publication Classification

(51) **Int. Cl.**[7] **A61K 48/00**; A61K 39/12

(52) **U.S. Cl.** **424/204.1**; 424/93.2; 424/780

(57) **ABSTRACT**

Compositions and methods for utilizing spore systems for medicinal and industrial protein applications are provided. Compositions comprise spores that produce and/or display carbohydrates, proteins, peptides, and nucleic acids of interest. Such spores are useful as therapeutic or prophylactic agents or vaccines against a broad spectrum of immunogens and bacterial and viral pathogens. Additionally, spore systems are useful in production, packaging, delivery, and presentation of polypeptides and/or nucleic acids for industrial catalysts, medical applications, and diagnostic applications.

DOCUMENT-IDENTIFIER: US 5683700 A
TITLE: Expression of recombinant proteins in attenuated bacteria

CLAIMS:

7. The method according to claim 6, wherein the heterologous protein is selected from the group consisting of the P.69 protein from Bordetella pertussis and tetanus toxin fragment C.

DOCUMENT-IDENTIFIER: US 20040171065 A1
TITLE: Method for expression of proteins on spore surface

CLAIMS:

23. The method according to any one of claims 1-5, wherein the protein of interest is selected from the group consisting of enzyme, enzyme inhibitor, hormone, hormone analogue, hormone receptor, signal transduction protein, antibody, monoclonal antibody, antigen, adhesive protein, structural protein, regulatory protein, toxin protein, cytokine, transcription regulatory protein, blood clotting protein, plant protection-inducing protein and fragments thereof.

24. The method according to any one of claims 1-9, wherein the host cell is selected from the group consisting of a spore-forming Gram negative bacterium including Myxococcus, a spore-forming Gram positive bacterium including Bacillus, a spore-forming Actionmycete, a spore-forming yeast or a spore-forming fungus.

26. The method according to claim 25, wherein the host cell is Bacillus.

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US 20030215920A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0215920 A1

Cregg (43) Pub. Date: Nov. 20, 2003

(54) FORMALDEHYDE DEHYDROGENASE GENES FROM METHYLOTROPHIC YEASTS

(76) Inventor: **James M. Cregg**, Claremont, CA (US)

Correspondence Address:
SCULLY SCOTT MURPHY & PRESSER, PC
400 GARDEN CITY PLAZA
GARDEN CITY, NY 11530

(21) Appl. No.: **10/459,970**

(22) Filed: **Jun. 12, 2003**

Related U.S. Application Data

(62) Division of application No. 09/345,828, filed on Jul. 2, 1999.

(60) Provisional application No. 60/091,699, filed on Jul. 3, 1998.

Publication Classification

(51) **Int. Cl.**[7] **C12P 21/02**; C07H 21/04; C12N 1/18; C12N 9/02; C12N 15/74

(52) **U.S. Cl.** **435/69.1**; 435/189; 435/254.2; 435/320.1; 536/23.2

(57) **ABSTRACT**

The present invention provides formaldehyde dehydrogenase genes (FLD) from methylotrophic yeasts. The FLD structural genes confer resistance to formaldehyde and are therefore useful as a selectable marker in methylotrophic yeasts. The FLD promoter sequences are strongly and independently induced by either methanol as sole carbon source (with ammonium sulfate as nitrogen source) or methylamine as sole nitrogen source (with glucose as carbon source). Induction under either methanol, methylamine or both provides levels of heterologous gene expression comparable to those obtained with the commonly used alcohol oxidase I gene promoter (P_{AOX1}). The FLD promoter of *Pichia pastoris* (P_{FLD1}) is an attractive alternative to P_{AOX1} for expression of foreign genes in *P. pastoris*, allowing regulation by carbon (methanol) or nitrogen (methylamine) source within the same expression strain. Yeast strains, expression cassettes, expression vectors, and host cells comprising an FLD gene promoter and 3' termination sequence are also provided.

DOCUMENT-IDENTIFIER: US 20030215920 A1
TITLE: Formaldehyde dehydrogenase genes from methylotrophic yeasts

CLAIMS:

34. The expression cassette of claim 33 wherein the heterologous gene is human serum albumin, invertase, bovine lysozyme, human EGF, mouse EGF, aprotinin, Kunitz protease inhibitor, Hepatitis B surface antigen, tumor necrosis factor, tetanus toxin fragment C, pertussis antigen P69, streptokinase, .beta.-galactosidase, or Bacillus sp. crystal protein toxin.

DOCUMENT-IDENTIFIER: US 20030158136 A1
TITLE: Materials and methods relating to immune responses to fusion proteins

CLAIMS:

11. A method of producing a nucleic acid construct for inducing an immune response in a patient, said method comprising (a) identifying a nucleic acid sequence encoding a disease peptide antigen comprising epitopes characteristic of the disease; (b) cloning the nucleic acid sequence encoding the disease peptide antigen; and (c) introducing the cloned nucleic acid into a vector, which vector allows for the disease peptide antigen to be expressed as a fusion with a first domain of FrC from tetanus toxin.

13. A method according to claim 11 or claim 12 further comprising the step of isolating a nucleic acid construct from the vector, said nucleic acid construct directing the expression of the fusion protein consisting of the first domain of FrC from tetanus toxin and the disease peptide antigen.

. A method of producing a nucleic acid construct for inducing an immune response in a patient, said method comprising (a) identifying a nucleic acid sequence encoding a disease peptide antigen comprising epitopes characteristic of the disease; (b) cloning the nucleic acid sequence encoding the disease peptide antigen; and (c) introducing the cloned nucleic acid into a vector, which vector allows for the disease peptide antigen to be expressed as a fusion with a first domain of FrC from tetanus toxin.

12. A method according to claim 11 wherein the vector further comprises a leader sequence for association with the fusion protein.

13. A method according to claim 11 or claim 12 further comprising the step of isolating a nucleic acid construct from the vector, said nucleic acid construct directing the expression of the fusion protein consisting of the first domain of FrC from tetanus toxin and the disease peptide antigen.

First Hit Fwd Refs

L10: Entry 41 of 53 File: USPT Dec 3, 2002

US-PAT-NO: 6488926
DOCUMENT-IDENTIFIER: US 6488926 B1

TITLE: Vaccine compositions

DATE-ISSUED: December 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Khan; Mohammed Anjam	Newcastle upon Tyne			GB
Hormaeche; Carlos Estenio	Newcastle upon Tyne			GB
Chatfield; Steven Neville	London			GB
Dougan; Gordon	London			GB

US-CL-CURRENT: 424/93.1; 424/184.1, 424/193.1, 424/197.11, 435/252.3, 435/320.1, 435/325, 530/350, 530/402, 536/23.4

CLAIMS:

what is claimed is:

1. A DNA construct comprising a DNA sequence encoding a fusion protein of the formula TetC-(Z).sub.a -Het, wherein TetC is the C fragment of tetanus toxin, Het is a heterologous protein, Z is an amino acid, and a is 0 or a positive integer less than 4, provided that (Z).sub.a does not include the sequence Gly-Pro, wherein the amino terminus of the fusion protein is the TetC.

2. A DNA construct according to claim 1 wherein (Z).sub.a is a chain of two or three amino acids, the DNA sequence for which defines a restriction endonuclease cleavage site.

3. A DNA construct according to claim 1 wherein a is zero.

4. A DNA construct according to claim 1 in which (Z).sub.a is free from glycine and/or proline.

5. A DNA construct according to claim 1 wherein the heterologous protein Het is an antigenic sequence obtained from a virus, bacterium, fungus, yeast or parasite.

6. A DNA construct according to claim 5 wherein the heterologous protein Het is the Schistosoma mansoni P28 glutathione S-transferase antigen.

7. A replicable expression vector containing a DNA construct as defined in claim 1.

8. A replicable expression vector according to claim 7 suitable for use in bacteria.

9. A host cell having integrated into the chromosomal DNA thereof a DNA construct as defined in claim 1.

10. A host cell according to claim 9 which is a bacterium.

11. A fusion protein of the formula TetC-(Z).sub.a -Het, wherein TetC is the C fragment of tetanus toxin, Het is a heterologous protein, Z is an amino acid, and a is 0 or a positive integer less than 4, provided that (Z).sub.a does not include the sequence Gly-Pro, wherein the amino terminus of the fusion protein is the TetC.

12. A process for the preparation of a bacterium which process comprises transforming a bacterium with a DNA construct as defined in claim 1.

13. A process according to claim 12 wherein the bacterium is an attenuated bacterium.

14. A vaccine composition comprising a fusion protein as defined in claim 11 and a pharmaceutically acceptable carrier.

15. A vaccine composition comprising an attenuated bacterium expressing a fusion protein as defined in claim 2; and a pharmaceutically acceptable carrier.

16. A replicable expression vector containing a DNA construct as defined in claim 2.

17. A replicable expression vector according to claim 16 suitable for use in bacteria.

18. A host cell having integrated into the chromosomal DNA thereof a DNA construct as defined in claim 2.

19. A host cell according to claim 18 which is a bacterium.

20. A vaccine composition comprising an attenuated bacterium containing a DNA construct as defined in claim 2; and a pharmaceutically acceptable carrier.

21. A fusion protein of the formula TetC-(Z).sub.a -Het, wherein TetC is the C fragment of tetanus toxin, Het is a heterologous protein, (Z).sub.a is a chain of two or three amino acids, the DNA sequence for which defines a restriction endonuclease cleavage site, wherein the amino terminus of the fusion protein is the TetC.

22. A vaccine composition containing a fusion protein as defined in claim 21 and a pharmaceutically acceptable carrier.

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First Hit

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L4: Entry 4 of 7 File: PGPB Sep 4, 2003

DOCUMENT-IDENTIFIER: US 20030165538 A1
TITLE: Methods and compositions for developing spore display systems for medicinal and industrial applications

Summary of Invention Paragraph:
[0005] Under conditions of a limitation in the supply of carbon, nitrogen, or phosphorous, certain gram-positive rods (aerobic Bacilli and anaerobic Clostridia) and a few sarcinae and actinomycetes form highly resistant, dehydrated forms called endospores or spores. Many gram-positive bacteria share the ability to form such a distinctive type of dormant cell. Bacterial spores can be readily recognized microscopically by their intracellular site of formation, their extreme refractility, and their resistance to staining by basic aniline dyes that readily stain vegetative cells. Other organisms are also capable of forming spores. For example, yeasts, such as the yeast Saccharomyces cerevisiae, form spores.

Detail Description Paragraph:
[0093] The spore systems of the present invention may be used with any organism that is capable of forming spores (e.g., bacteria or fungus such as, for example, yeast and the like). Thus, any of a variety of spore-forming organisms may be useful in practicing the methods and compositions of the present invention. For example, spore-forming bacteria which may be useful in practicing the present invention include, but are not limited to, Clostridium botulinum, Clostridium lentoputrescens, Clostridium perfringens, Clostridium sporogenes, Clostridium tetuni, and Bacillus species, for example Bacillus anthracis, Bacillus coagulans, Bacillus globigii, Bacillus stearothermophilus, Bacillus thuringiensis, and Bacillus subtilis.

Detail Description Paragraph:
[0175] In one aspect, a spore system of the invention may function as a therapeutic, prophylactic, or immunomodulatory agent or vaccine against a disease or disease-inducing pathogen, including but not limited to, Yersinia pestis, Staphylococcus aureus, Streptococcus pyogenes, viral encephalitis, Herpes simplex virus Type 1, Herpes simplex virus Type 2, Varicella-zoster virus (Herpes zoster), cytomegalovirus, poliomyelitis, rabies, cancer, typhoid, parasites (including, e.g., parasites associated with malaria, African sleeping sickness, Giardia), typhus, anthrax, foot and mouth disease, HIV, pertussis, diphtheria, Ebola, hemorrhagic fevers, influenza, smallpox, cholera, dengue fever, measles, mumps, German measles, chicken pox, hepatitis A, hepatitis B, hepatitis C, Alzheimer's, human papillomavirus, meningitis, mononucleosis, Lyme disease, tetanus, Rocky Mountain spotted fever, Salmonella, Yellow fever, pneumonia, Mycobacterium tuberculosis, Respiratory Syncytial Virus, Creutzfield-Jacob Disease, Chlamydia trachomatis, syphilis, Listeria monocytogenes, Gonorrhea, Parvovirus, Paramyxoviridae diseases, Coxsackievirus, Rhinovirus, hantavirus, Japanese encephalitis, Eastern equine encephalitis, Western equine encephalitis, tick-borne encephalitis, West Nile Encephalitis, and Legionella pneumophila, bacterial enterotoxigenic strains of E. coli (e.g., heat-labile toxin from E. coli), and salmonella toxin, shigella toxin and Campylobacter toxin. Spore systems comprising antigens or antigenic peptides associated with such diseases or toxins (e.g., having antigens expressed or displayed on the spore surface) can be prepared in any

of the formats described herein and used in the therapeutic or prophylactic methods described herein.

CLAIMS:

1. A method for modulation of an immune response of an organism, said method comprising contacting said organism with a spore system comprising a recombinant spore having at least one exogenous nucleic acid, peptide, or polypeptide which modulates an immune response in the organism, wherein said spore is administered via a delivery system selected from the group consisting of respiratory delivery system, nasal delivery system, parenteral delivery system, and mucosal delivery system.

4. The method of claim 1, wherein the nucleic acid, peptide, or polypeptide is displayed on or bound to a surface of the spore.

5. The method of claim 1, wherein the nucleic acid, peptide, or polypeptide is contained within the spore.

6. The method of claim 1, wherein said modulation results from the release of the nucleic acid, peptide, or polypeptide from the spore system.

7. The method of claim 1, wherein the spore of said spore system is a non-viable spore.

8. The method of claim 1, wherein the spore of said spore system is a bacterial spore.

10. The method of claim 9, wherein said at least one immunomodulatory agent is selected from the group consisting of: cytokines, co-stimulatory agents, antigens, antibodies, adjuvants, and binding receptors.

12. The method of claim 1, wherein the polypeptide or peptide is produced as a fusion protein with a spore coat protein.

13. The method of claim 12, wherein the spore coat protein is at least one protein selected from the group consisting of: CotA, CotB, CotC, CotD, CotE, CotF, CotG, CotN, CotS, CotT, CotV, CotW, CotX, CotY, and CotZ.

14. The method of claim 13, wherein the spore coat protein is CotC protein.

15. The method of claim 13, wherein the spore coat protein is CotD protein.

16. The method of claim 1, wherein the nucleic acid, peptide, or polypeptide is produced by vegetative cells produced by said spore following germination of said spore.

17. The method of claim 1, wherein said spore is delivered via the respiratory delivery system.

18. The method of claim 1, wherein said spore is delivered via the nasal delivery system.

19. The method of claim 1, where said spore is delivered via the parenteral delivery system.

20. The method of claim 1, wherein said polypeptide is displayed on the surface of the spore after lysis of the mother cell of said spore.

21. A method for modulation of an immune response of an organism, said method

comprising contacting said organism with a spore system comprising a non-viable recombinant spore having at least one exogenous nucleic acid, peptide, or polypeptide which modulates an immune response in the organism.

22. The method of claim 21, wherein said nucleic acid, peptide, or polypeptide is displayed on or bound to a surface of the spore.

24. The method of claim 21, wherein said polypeptide, or peptide is produced as a fusion protein with a spore coat protein.

25. The method of claim 24, wherein said spore coat protein is at least one protein selected from the group consisting of: CotA, CotB, CotC, CotD, CotE, CotF, CotG, CotN, CotS, CotT, CotV, CotW, CotX, CotY, CotZ.

26. A composition comprising a spore system, said spore system comprising a spore and at least one exogenous nucleic acid molecule, peptide, or polypeptide displayed on, bound to, or contained within said spore wherein said nucleic acid, peptide, or polypeptide modulates an immune response when administered to an organism via the respiratory delivery system, nasal delivery system, or the parenteral delivery system.

27. The composition of 26, wherein the nucleic acid, peptide, or polypeptide is displayed on or bound to the surface of the spore.

28. The composition of 26, wherein the peptide or polypeptide is produced as a fusion protein with a spore coat protein.

29. The composition of 28, wherein the spore coat protein is at least one protein selected from the group consisting of: CotA, CotB, CotC, CotD, CotE, CotF, CotG, CotN, CotS, CotT, CotV, CotW, CotX, CotY, and CotZ.

30. A composition comprising a spore system, said spore system comprising a spore, at least one antigen, and at least one adjuvant and/or co-stimulatory polypeptide.

31. The composition of claim 30, wherein said at least one antigen is displayed on or bound to the surface of the spore.

32. The composition of claim 30, wherein said at least one antigen is contained within the spore.

33. The composition of claim 30, wherein said spore of said spore system is a non-viable spore.

34. The composition of claim 30, wherein said at least one adjuvant or co-stimulatory polypeptide is displayed on, bound to, or contained within said spore.

36. The composition of claim 30, wherein said at least one antigen is a fusion protein comprising a spore coat polypeptide.

37. The composition of claim 30, wherein said at least one adjuvant is a fusion protein comprising a spore coat polypeptide.

38. The composition of claim 30, wherein said at least one co-stimulatory polypeptide is a fusion protein comprising a spore coat polypeptide.

42. A method for releasing a spore system of interest, said method comprising: a) transforming a cell that is capable of sporulation with an exogenous nucleic acid molecule; b) inducing sporulation of the cell, whereby at least one spore system is produced, said spore system comprising said nucleic acid molecule and/or any polypeptide produced therefrom, and a spore; and c) lysing the cell to release said

spore system.

44. The method of claim 43, wherein said polypeptide is displayed on or bound to a surface of the spore.

45. The method of claim 43, wherein said polypeptide is contained within said spore.

46. The method of claim 43, wherein the polypeptide is a fusion protein comprising a spore coat protein of the spore.

47. The method of claim 42, wherein said cell that is capable of sporulation is a bacterial cell.

48. The method of claim 42, wherein said cell that is capable of sporulation is a fungal cell.

50. A method for displaying a polypeptide at one or more sites of interest on a surface of a spore, said method comprising: a) transforming a cell that is capable of sporulation with a recombinant nucleic acid vector comprising a nucleic acid molecule encoding a polypeptide fused in frame to a nucleic acid molecule encoding a spore coat protein; and b) expressing a fusion protein comprising said polypeptide and said spore coat protein such that said fusion protein is attached to the spore coat of the spore at one or more sites of interest on the surface of the spore.

51. The method of claim 50, wherein said spore coat protein is selected from the group consisting of: CotA, CotB, CotC, CotD, CotE, CotF, CotG, CotN, CotS, CotT, CotV, CotW, CotX, CotY, and CotZ.

52. A detection system comprising a spore system wherein said spore system comprises a moiety that provides a detectable signal and a polypeptide displayed on, bound to, or contained within the spore system, wherein said polypeptide is capable of capturing a detectable compound.

57. A method for delivery of a polypeptide of interest, said method comprising: a) transforming a cell that is capable of sporulating with a nucleic acid sequence encoding said polypeptide; b) inducing sporulation of said cell to form a spore; and c) delivering said spore to a site of interest.

58. The method of claim 57, wherein said polypeptide is displayed on or bound to the surface of said spore.

59. The method of claim 57, wherein said polypeptide is contained within said spore.

60. The method of claim 57, wherein said spore is delivered as an intact spore.

61. The method of claim 57, wherein said spore is delivered as a germinated spore.

62. The method of claim 57, wherein said spore is delivered as a replicating vegetative cell arising from a spore.

63. The method of claim 57, wherein said polypeptide is expressed as a fusion protein with a spore coat protein.

64. The method of claim 63, wherein said spore coat protein is selected from the group consisting of: CotA, CotB, CotC, CotD, CotE, CotF, CotG, CotN, CotS, CotT, CotV, CotW, CotX, CotY, and CotZ.

65. A method for generating a desired product comprising reacting a substrate with a spore system, said spore system comprising a recombinant spore having at least one polypeptide wherein said polypeptide has enzymatic activity.

66. The method of claim 65, wherein said spore comprises the enzymes needed to produce said desired product.

67. A composition comprising a spore system said spore system comprising a recombinant spore having at least one exogenous polypeptide wherein said polypeptide has enzymatic activity and a substrate wherein said enzyme alters said substrate.

68. A composition comprising a spore system, said spore system comprising a non-viable spore and at least one exogenous nucleic acid, peptide, or polypeptide displayed on, bound to, or contained within said spore.

69. The composition of claim 68, wherein the nucleic acid, peptide, or polypeptide is displayed on or bound to the surface of the spore.

70. The composition of claim 68, wherein the nucleic acid, peptide, or polypeptide is contained within the spore.

71. The composition of claim 68, wherein said spore system comprises more than one exogenous nucleic acid, peptide, or polypeptide associated with said spore.

74. The composition of claim 68, wherein said spore system is immobilized by attachment to a solid support.

79. A composition comprising a spore system, said spore system comprising a non-viable spore and one or more expression cassettes, wherein said one or more expression cassettes comprise a promoter operably linked to a nucleotide sequence of interest.

80. A composition of claim 79, wherein said nucleotide sequence of interest is operably linked to a nucleotide sequence encoding a spore coat protein.

81. A composition of claim 80, wherein said spore coat protein is selected from the group consisting of CotA, CotB, CotC, CotD, CotE, CotF, CotG, CotN, CotS, CotT, CotV, CotW, CotX, CotY, and CotZ.

82. A composition of claim 79, wherein the polypeptide encoded by the nucleotide sequence of interest is targeted to the spore coat.

83. A method for modulation of an adjuvant effect in an organism, said method comprising: a) generating a non-viable spore, wherein said spore has an adjuvant effect; b) isolating said spore; and c) contacting said organism with said spore and a nucleic acid, peptide, or polypeptide.

84. The method of claim 83, wherein said spore is a recombinant spore.

85. The method of claim 83, wherein said spore is a non-recombinant spore.

86. A composition comprising a spore system, said spore system comprising a non-viable spore and one or more expression cassettes, wherein said expression cassettes are comprised of a promoter operably linked to a multiple cloning site.

87. A composition of claim 86, wherein said multiple cloning site is operably linked to a nucleotide sequence encoding a spore coat protein.

88. A composition comprising a spore system, said spore system comprising a spore

and at least one streptavidin or avidin molecule displayed on or bound to said spore.

89. The composition of claim 88, wherein said spore of said spore system is a non-viable spore.

92. The composition of claim 88, wherein said streptavidin or avidin molecule is a fusion protein with a spore coat protein.

93. A composition comprising a spore system, said spore system comprising a spore and at least one exogenous nucleic acid binding particle displayed on or bound to said spore.

94. The composition of claim 93, wherein said spore of said spore system is non-viable.

97. The composition of claim 93, wherein said nucleic acid binding particle is a fusion protein comprising a spore coat protein.

98. A composition comprising a spore system, said spore system comprising a spore and at least one peptide, polypeptide, protein, carbohydrate, or nucleotide sequence having anti-pathogenic activity displayed on, bound to, or contained within said spore.

99. The composition of claim 98, wherein said spore of said spore system is non-viable.

100. The composition of claim 98, wherein said peptide, polypeptide, or protein having anti-pathogenic activity is a fusion protein comprising a spore coat protein.

101. A method of enhancing an immune response to an immunogenic polypeptide or peptide in a subject, said method comprising administering to the subject a population of spores and an expression vector comprising a nucleotide sequence encoding the immunogenic polypeptide or peptide, wherein the immune response is enhanced compared to the immune response generated by administration of the expression vector or encoded immunogenic polypeptide or peptide alone to the subject.

104. The method of claim 101, wherein the population of spores comprises non-viable or non-germinating spores.

105. The method of claim 101, wherein the spores have an adjuvant effect.

106. A method of enhancing an immune response to an immunogenic polypeptide or peptide in a subject, said method comprising administering to the subject a population of spores and an immunogenic polypeptide or peptide, wherein the immune response to the immunogenic polypeptide or peptide is enhanced compared to the immune response generated by administration of the immunogenic polypeptide or peptide alone to the subject.

109. The method of claim 106, wherein the population of spores comprises non-viable or non-germinating spores.

110. The method of claim 106, wherein the spores act as adjuvants to enhance the immune response.

111. A composition comprising a spore system, said spore system comprising at least two spores wherein each spore displays a different peptide, polypeptide, or protein.

112. The composition of claim 111, wherein said spore is non-viable.

113. The composition of claim 111, wherein said polypeptide is a fusion protein comprising a spore coat protein.

115. The composition of claim 111, wherein said spore system modulates more than one immune response in an organism.

117. A composition comprising a spore system, said spore system comprising a spore and at least one rotavirus capsid protein displayed on, bound to, or contained within said spore.

120. The composition of claim 117, wherein said spore is non-viable.

121. The composition of claim 117, wherein said spore system modulates an immune response when administered to an organism.

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Referring to FIG. 5, a strong sporulation promoter was placed in front of Apro* as follows. The spoVG promoter (also known as the 0.4 kb gene promoter), a strong sporulation promoter described in Moran et al. (1981) Cell 25, 783-791, was isolated as a 1.7 kb EcoRI-XbaI fragment from E. coli plasmid pAL1. The sequence of this promoter is given in Moran et al., id. This fragment was ligated with SpeI-cut pApro*-tet. SpeI cuts pApro*-tet approximately 100 bp before the start of the Apro* gene. The ligated DNA was then used to transform the B. subtilis strain having the amylase-APIII fusion chromosomally integrated, as described above (strain GP203). Plasmid DNA from Tet, colonies was isolated, and one plasmid, pAL2, was found to have the spoVG promoter in the right orientation 5' to the Apro* gene. This colony also produced the largest halo on starch-azure indicator plates.

ribosomal rRNA operon promote

DOCUMENT-IDENTIFIER: US 20020048816 A1
TITLE: EXPRESSION OF SURFACE LAYER PROTEINS

CLAIMS:

1- A host cell which is provided with a S-layer comprising a fusion polypeptide having: (a) at least sufficient of a S-layer protein for a S-layer composed thereof to assemble, and (b) a heterologous polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell.

2- A cell according to claim 1 which is a bacterium of the genus Bacillus.

3- A cell according to claim 2 wherein the bacterium is B. sphaericus P-1 (LMG P-13855).

4- A cell according to claim 1, wherein the heterologous polypeptide is fused to either the carboxy terminus or the amino terminus of the most N-terminal 41% or more amino acid residues of a S-layer protein.

5- A cell according to claim 1, wherein the S-layer protein is derived from B. sphaericus.

6- A cell according to claim 1, wherein the heterologous polypeptide is an antigenic peptide.

7- A cell according to claim 6, wherein the heterologous polypeptide comprises an antigenic determinant of a pathogen selected from a virus, bacterium, fungus, yeast and parasite.

8- A cell according to claim 6, wherein the heterologous polypeptide is selected from the group consisting of P69 antigen of Bordetella pertussis, pertussis toxin, a subunit of pertussis toxin, tetanus toxin fragment C, E. coli heat labile toxin B subunit and an E. coli K88 antigen.

9- Sacculi derived from a host cell according to claim 1.

10- A pharmaceutical or veterinary composition comprising the host cell of claim 1 and a pharmaceutically or veterinarily acceptable carrier or diluent.

11- A vaccine comprising the host cell of claim 6 and an acceptable carrier or diluent therefor.

12- A recombinant DNA molecule comprised of a promoter operably linked to a coding sequence which encodes a signal peptide and a fusion polypeptide, the signal peptide being capable of directing the said fusion polypeptide to be presented on the surface of a host cell in which expression occurs and the fusion polypeptide being of a heterologous polypeptide fused to either the carboxy terminus or the amino terminus of at least sufficient of a S-layer protein for a S-layer composed thereof to assemble.

13- A molecule according to claim 12, wherein the promoter is a promoter for a S-layer protein from a Bacillus bacterium.

14- A molecule according to claim 13, wherein the promoter is the P1 promoter of B. sphaericus P-1 (LMG P-13855).

15- A molecule according to claim 12, wherein the signal peptide is the signal peptide for the S-layer protein of which an appropriate portion is incorporated in the fusion polypeptide.

16- A molecule according to claim 12, wherein the signal peptide is a signal peptide for a S-layer protein of a Bacillus bacterium.

17- A molecule according to claim 12 which is an expression vector.

18- A molecule according to claim 17, wherein the vector is a plasmid.

19- A host cell having the recombinant DNA molecule of claim 12.

20- A host cell according to claim 19 which has been transformed with the vector of claim 17.

21- A cell according to claim 19 which is a gram-positive bacterium.

22- A cell according to claim 19 which is a bacterium of the genus Bacillus.

23- A cell according to claim 22 wherein the bacterium is B. sphaericus P-1 (LMG P-13855).

24- A process for the preparation of a host cell provided with a S-layer comprising a fusion polypeptide, which process comprises: (i) providing a suitable host cell incorporating a recombinant DNA molecule having a promoter operably linked to a coding sequence which encodes a signal peptide and a fusion polypeptide, the signal peptide being capable of directing the said fusion polypeptide to be presented on the surface of the said host cell and the fusion polypeptide being a heterologous polypeptide fused to either the carboxy terminus or the amino terminus of at least sufficient of a S-layer protein for a S-layer composed thereof to assemble; and (ii) culturing the said host cell so that the said fusion polypeptide is expressed and a S-layer having the fusion polypeptide is formed on the surface of the said host cell, the heterologous polypeptide thereby being presented on the outer surface of the said host cell.

25- A process according to claim 24, which comprises: (a) providing an intermediate vector in which the coding sequence of an internal portion of the native S-layer protein of the said host cell is translationally fused to the 3'-end thereof the coding sequence for the heterologous polypeptide and in which the said coding sequences are provided upstream of a promotorless selectable marker gene such that they form a translational or transcriptional fusion therewith; (b) transforming the said host cell with the intermediate vector; (c) selecting a transformed host cell which has a S-layer comprising the said fusion polypeptide.

26- A process according to claim 24 which comprises: (a) fusing to a promoter a S-layer protein coding sequence coding for the signal peptide and at least sufficient of the amino-terminal portion of a S-layer protein for a S-layer composed thereof to assemble on the surface of the host cell, and fusing a peptide coding sequence coding for the heterologous polypeptide to the 3'-end of the S-layer protein coding sequence, whereby a recombinant DNA molecule for the expression and presentation of the fusion polypeptide is prepared; (b) inserting the recombinant DNA molecule into a suitable vector, whereby a recombinant DNA vector is prepared; (c) transforming a suitable host cell with the recombinant DNA vector, whereby a transformed host cell having the recombinant DNA molecule is provided; and (d) culturing the transformed host cell, whereby the fusion polypeptide is expressed and a S-layer comprising the fusion polypeptide is assembled on the host cell wall.

27- A promoter having a -35 region of the sequence TTGAAT and a -10 region of the sequence TATATT.

28- A promoter according to claim 27, having the sequence CTAAATTATGTCCCAATGCTTGAATTTCGGAAAAGATAGTGTTAT ATTATTGT.

29- A promoter having a -35 region of the sequence CTTGGTT and a -10 region of the sequence TATAAT.

30- A promoter according to claim 29, having the sequence TCCAGAAAATGCTTGGTTATTATTGAGAGTAAGGTATAATAGGTA.

31- A promoter having a -35 region of the sequence ATTACGGGA and a -10 region of the sequence TTTAGT.

32- A promoter according to claim 31, having the sequence AAAATATTACGGGAGTCTTTAATTTTGACAATTTAGTAACCAT.

33- The promoter according to claim 27, having the sequence from nucleotide 52 to 353 shown in FIG. 10.

34- The promoter according to claim 29, having the sequence from nucleotide 52 to 353 shown in FIG. 10.

35- The promoter according to claim 31, having the sequence from nucleotide 52 to 353 shown in FIG. 10.

36- An expression vector comprised of a promoter as defined in any one of claim 27 and a downstream cloning site into which a DNA sequence encoding a heterologous protein may be cloned such that the promoter is operably linked to the said sequence.

37- An expression vector comprised of a promoter as defined in any one of claim 29 and a downstream cloning site into which a DNA sequence encoding a heterologous protein may be cloned such that the promoter is operably linked to the said sequence.

38- An expression vector comprised of a promoter as defined in any one of claim 31 and a downstream cloning site into which a DNA sequence encoding a heterologous protein may be cloned such that the promoter is operably linked to the said sequence.

39- An expression vector having a promoter as defined in any one of claim 27 operably linked to a DNA sequence encoding a heterologous protein.

40- An expression vector having a promoter as defined in any one of claim 29 operably linked to a DNA sequence encoding a heterologous protein.

41- An expression vector having a promoter as defined in any one of claim 31 operably linked to a DNA sequence encoding a heterologous protein.

42- A DNA fragment comprising a promoter according to any one of claims 27 operably linked to a DNA sequence encoding a heterologous protein.

43- A DNA fragment comprising a promoter according to any one of claims 29 operably linked to a DNA sequence encoding a heterologous protein.

44- A DNA fragment comprising a promoter according to any one of claims 31 operably linked to a DNA sequence encoding a heterologous protein.

45- A host cell transformed with an expression vector as defined in claim 39.

46- A host cell transformed with an expression vector as defined in claim 40.

47- A host cell transformed with an expression vector as defined in claim 41.

48- A process for the preparation of a heterologous protein, which process comprises culturing a transformed host cell according to claim 45 and obtaining the heterologous protein thus expressed.

49- A process for the preparation of a heterologous protein, which process comprises culturing a transformed host cell according to claim 46 and obtaining the heterologous protein thus expressed.

50- A process for the preparation of a heterologous protein, which process comprises culturing a transformed host cell according to claim 47 and obtaining the heterologous protein thus expressed.

51- A pharmaceutical or veterinary composition comprising a pharmaceutically or veterinarily acceptable carrier or diluent and, as active ingredient, a physiologically active heterologous protein which has been obtained by the process of claim 48.

52- A pharmaceutical or veterinary composition comprising a pharmaceutically or veterinarily acceptable carrier or diluent and, as active ingredient, a physiologically active heterologous protein which has been obtained by the process of claim 49.

53- A pharmaceutical or veterinary composition comprising a pharmaceutically or veterinarily acceptable carrier or diluent and, as active ingredient, a physiologically active heterologous protein which has been obtained by the process of claim 50.

54- A process of transforming B. sphaericus P-1 cells with DNA, which process comprises harvesting B. sphaericus P-1 cells at the late stationary growth phase, mixing the harvested cells with the DNA and effecting electroporation to cause entry of the DNA into the said cells.

55- Use of the host cell of claim 1 for immobilisation purposes.

56- Use of the host cell of claim 1 for screening purposes.

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DOCUMENT-IDENTIFIER: US 6881558 B1

TITLE: Expression system for cloning toxic genes

DATE-ISSUED: April 19, 2005

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/91.1; 435/252.1, 435/252.3, 435/320.1, 435/325, 435/348, 435/455, 435/69.1, 536/23.1, 536/24.1, 536/24.2

CLAIMS:

What is claimed is:

1. A vector for amplifying a toxic gene in bacteria comprising: an origin of replication; a first promoter; a polylinker; a lac promoter in reverse orientation with respect to said first promoter; a polyadenylation signal; and a nucleic acid molecule having a nucleotide sequence encoding a selectable marker; wherein said lac promoter is capable of producing an antisense molecule directed to said toxic gene when a nucleotide sequence encoding a toxic gene product inserted into said polylinker of said vector.

2. A vector for amplifying a toxic gene in bacteria comprising: an origin of replication; a first promoter; a polylinker; a nucleic acid molecule having a nucleotide sequence encoding a toxic protein, wherein said nucleic acid molecule is inserted within said polylinker and is operably connected to said first promoter; a second promoter in reverse orientation with respect to said first promoter; a polyadenylation signal; and a nucleic acid molecule having a nucleotide sequence encoding a selectable marker; wherein said second promoter is capable of producing an antisense molecule directed to said nucleic acid molecule encoding a toxic protein.

3. The vector of claim 2 wherein said nucleic acid molecule encoding a toxic protein encodes a bacterial toxin or a viral toxin.

4. The vector of claim 3 wherein said viral toxin is HIV-1 env.

5. The vector of claim 3 wherein said bacterial toxin is selected from the group consisting of Pseudomonas exotoxin A, cholera toxin, diphtheria toxin. E. coli toxins, botulinum toxin, anthrax toxin, pertussis toxin, shiga toxin, ricin, tetanus toxin, and Staphylococcal toxins.

6. A method of amplifying a toxic gene in bacteria comprising the steps; providing a vector of claim 2; inserting said vector comprising said toxic gene into said bacteria; and amplifying said vector in said bacteria.

7. A bacterial cell comprising the vector of claim 2.

8. A mammalian cell comprising the vector of claim 2.

Entry 40 of 53 File: USPT May 4, 2004

US-PAT-NO: 6730499
DOCUMENT-IDENTIFIER: US 6730499 B1

TITLE: Promoter for the Pichia pastoris formaldehyde dehydrogenase gene FLD1

DATE-ISSUED: May 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cregg; James M.	Claremont	CA		

US-CL-CURRENT: 435/69.1; 435/243, 435/254.2, 435/255.1, 435/255.5, 435/320.1, 435/471, 435/479, 435/483, 435/69.2, 435/69.8, 536/23.1, 536/24.1

CLAIMS:

What is claimed is:

1. An expression cassette which comprises an isolated promoter sequence and a heterologous coding sequence, wherein said promoter sequence comprises the sequence as set forth in SEQ ID NO: 3 and is operably linked to the heterologous coding sequence.

2. The expression cassette of claim 1 wherein the heterologous coding sequence encodes human serum albumin, invertase, bovine lysozyme, human EGF, mouse EGF, aprotinin, Kunitz protease inhibitor, Hepatitis B surface antigen, tumor necrosis factor, tetanus toxin fragment C, pertussis antigen P69, streptokinase, .beta.-galactosidase, or Bacillus sp. crystal protein toxin.

3. The expression cassette of claim 1 further comprising a 3' termination sequence, wherein said 3' termination sequence is operably linked to the 3' end of said heterologous coding sequence.

4. The expression cassette of claim 3, wherein said 3' termination sequence is from an FLD gene of a methylotrophic yeast, and wherein said FLD gene comprises the coding sequence as set forth in SEQ ID NO: 5.

5. The expression cassette of claim 3 wherein the 3' termination sequence is that of the Pichia pastoris AOX1 gene, the Pichia pastoris p40 gene or the Pichia pastoris HIS4 gene.

6. An expression vector which comprises an expression cassette according to claim 1.

7. An expression vector which comprises an expression cassette according to claim 3.

8. An expression vector which comprises an expression cassette according to claim 4.

9. An expression vector which comprises an expression cassette according to

claim 5.

10. A host cell comprising an expression cassette according to claim 1.

11. A host cell comprising an expression cassette according to claim 3.

12. A host cell comprising an expression cassette according to claim 4.

13. A host cell comprising an expression cassette according to claim 5.

14. A host cell comprising an expression vector according to claim 6.

15. A host cell comprising an expression vector according to claim 7.

16. A host cell comprising an expression vector according to claim 8.

17. A host cell comprising an expression vector according to claim 9.

18. The host cell of claim 14 wherein said host cell is a methylotrophic yeast cell.

19. The host cell of claim 15 wherein said host cell is a methylotrophic yeast cell.

20. The host cell of claim 16 wherein said host cell is a methylotrophic yeast cell.

21. The host cell of claim 17 wherein said host cell is a methylotrophic yeast cell.

22. The host cell of claim 18 wherein the methylotrophic yeast cell is from the genus Pichia, Candida, Hansenula, or Torulopsis.

23. The host cell of claim 19 wherein the methylotrophic yeast cell is from the genus Pichia, Candida, Hansenula, or Torulopsis.

24. The host cell of claim 20 wherein the methylotrophic yeast cell is from the genus Pichia, Candida, Hansenula, or Torulopsis.

25. The host cell of claim 21 wherein the methylotrophic yeast cell is from the genus Pichia, Candida, Hansenula, or Torulopsis.

26. A method for directing expression of a heterologous coding sequence in a methylotrophic yeast which comprises: a) introducing into a methylotrophic yeast cell an isolated nucleic acid comprising an isolated promoter sequence and said heterologous coding sequence, wherein said promoter sequence comprises the sequence as set forth in SEQ ID NO: 3 and is operably linked at its 3' end to the 5' end of the heterologous coding sequence, said heterologous coding sequence operably linked at its 3' end to the 5' end of a termination sequence which functions in methylotrophic yeast; b) growing said methylotrophic yeast cell in a medium having a carbon source and a nitrogen source, and after the carbon or nitrogen source is depleted; c) inducing expression of said heterologous coding sequence by addition of methanol or methylamine or both methanol and methylamine.

27. A method for directing expression of a heterologous coding sequence in a methylotrophic yeast which comprises: a) introducing into a methylotrophic yeast cell an isolated nucleic acid comprising an isolated promoter sequence and said heterologous coding sequence, wherein said promoter sequence comprises the sequence as set forth in SEQ ID NO: 3 and is operably linked at its 3' end to the 5' end of the heterologous coding sequence, said heterologous coding sequence operably linked at its 3' end to the 5' end of a termination sequence which functions in methylotrophic yeast; b) growing said methylotrophic yeast cell in a medium having a carbon source and a nitrogen source, and after the carbon or nitrogen source is depleted; c) inducing expression of said heterologous coding sequence by addition of formaldehyde, formate, or a methylated amine.

28. The method of claim 27 wherein the methylated amine is choline.

29. A kit which comprises: a) an expression cassette comprising an isolated promoter sequence and a 3' termination sequence which functions in a methylotrophic yeast, wherein said promoter sequence comprises the sequence as set forth in SEQ ID NO: 3, and wherein at least one restriction site is located between said promoter sequence and said 3' termination sequence so that a heterologous coding sequence may be inserted and operably linked to said promoter sequence and said 3' termination sequence; and b) a vector which replicates in a methylotrophic yeast or which integrates into the genome of a methylotrophic yeast, said vector comprising a marker gene and one or more restriction sites for insertion of said expression cassette.

30. An isolated promoter sequence, wherein said promoter sequence comprises the sequence as set forth in SEQ ID NO: 3.

31. The method according to claim 26 or 27, wherein said carbon source is selected from glycerol or glucose, and wherein said nitrogen source is selected from ammonium salt or ammonium hydroxide.